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Growth hormone combined with testosterone helps normalize penile conditions in microphallic rats of hypogonadotropic hypogonadism

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Growth hormone combined with testosterone helps normalize penile conditions in microphallic rats of hypogonadotropic hypogonadism

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Abstract

Introduction: Hypogonadotropic hypogonadism has been known as the most common cause of micropenis. Until now, testosterone therapy is the only effective treatment option in the management of micropenis. The tropic effect of growth hormone (GH) on penis has not been studied. While the underlying molecular mechanism is unclear, the growth hormone / insulin-like growth factor-1 (IGF-1) axis possesses proactive effects for the action of testosterone and its potent derivative, dihydrotestosterone.

This animal study was aimed to evaluate the role of GH, alone or in conjunction with testosterone on phallic growth.

Methods: Seven timed-pregnant Sprague-Dawley rats were obtained one week before parturition. After parturition, pups remained with their mother until two weeks of age. Male pups were discriminated by females by longer anogenital length on the third postnatal day, although this was not always possible. Five study groups among male pups were assigned to control (C), microphallus (MP), testosterone (T), GH (G) and GH plus testosterone (GT). Microphallus was induced by secondary hypogonadism using leuprolide acetate injection. Pre-pubertal administration of testosterone, GH or combination of both was started from seven days after birth and maintained up to 12 weeks of age. Phallic dimensions and histological markers of cavernosal integrity were assessed as efficacy measure. To assess bone growth, adjusted penile lengths were calculated as the ratio of penile and right tibial lengths. Testicular weights were also compared for evaluating gonadal safety. Comparison of phallic dimensions and various cavernosal histological and molecular markers such as expressions of smooth muscle

actin, collagen Ⅰ and Ⅲ, the number of fat globules, sinusoidal density and expression of androgen receptor (AR) were made.

Results: Compared to C, MP showed significantly lower mean plasma level of testosterone. Treatment with either testosterone or GH resulted in corresponding increase in plasma levels. Comparing control group (C), microphallus (MP) showed prominent decrease of penile length, penile weight, testis volume, expressions of smooth muscle actin. Collagen Ⅰ and Ⅲ and the number of fat globules was shown significant increase in MP. No monotherapy successfully normalized all tested penile dimensions, though certain phallic dimensions were enhanced by treatment of T. Combination treatment (GT) led to complete normalization of all phallic dimensions. Also, this effectively prevented deterioration of cavernosal histological markers where microphallic rats showed significant changes. Ratios of penile length / right tibial length were similar to those of penile lengths, indicating minimal effects of the present treatments on bone growth.

GH administration led to enhanced AR expression in cavernosum and alleviation of testicular volume loss. GH administration with testosterone was effective measure to enhance penile growth by augmenting AR with minimizing gonadal volume loss. This proof of concept study could be helpful to circumvent the problem of using testosterone monotherapy in human micropenis. The different physiological background on phallic growth between human and rats may hinder the application of the result to clinical situations.

Conclusion: Our results showed that the combination therapy of testosterone with growth hormone might be further treatment option of micropenis related to

hypogonadotropic hypogonadism. The size and structural problems of microphallus could be effectively and safely addressed by combination treatment of growth hormone (GH) and testosterone.

Key words: rats, micropenis, testosterone, growth hormone

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Abbreviation

ANOVA:	Analysis of variance
AR:	Androgen receptor
ARv7:	Androgen receptor splicing variants
BSA:	Bovine serum albumin
cGMP:	cyclic guanosine monophosphate
DSD:	Disorders of sexual differentiation
ELISA:	Enzyme-linked immunosorbent assays
GH:	Growth hormone
HCG:	Human chorionic gonadotropin
IGF-1:	Insulin-like growth factor-1
LH:	Luteinizing hormone
PVDF:	Polyvinylidene difluoride
SMA:	Smooth muscle actin
T:	Testosterone
TBS-T:	Mixture of tris-buffered saline (TBS) and Polysorbate 20

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comparison was done for either of these variables.

Fig. 7 Immunohistochemical localizations of collagen III was compared among five experimental groups. Relative amount of each expressions was semiquantitatively measured. Scale bar indicates 100um. Quantitative evaluation of relative expression was depicted in densitogram. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

Fig. 8 Immunohistochemical localizations of smooth muscle actin was compared among five experimental groups. Relative amount of each expressions was semiquantitatively measured. Scale bar indicates 100um. Quantitative evaluation of relative expression was depicted in densitogram. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

Fig. 9 Quantitative evaluation of relative expression was depicted in densitogram of sinusoidal density. The sinusoidal density was also measured and described. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

Fig. 10 Quantitative evaluation of relative expression was depicted in densitogram of the number of fat globules. The number of fat globules was also measured and described. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

Contents

I. Introduction

Normal penis is defined as normal range of penile length and circumference. There are several studies to measure penile length and circumference for the assessment of normal penis (1,2).

The term micropenis encompasses a range of congenital and acquired conditions that result in an abnormally short penis with a stretched length of more than 2.5 SD below the mean for age and with a normal looking urethral meatus at the tip of the glans penis, i.e., no hypospadias. An isolated micropenis is commonly associated with testosterone deficiency after 12 weeks of gestation, suggesting normal placental human chorionic gonadotropin (HCG)-induced testosterone levels during the period of organogenesis (3). Thus, hypogonadotropic hypogonadism due to deficient fetal LH function is more frequent than hypergonadotropic hypogonadism, which is often associated with stigmata of disorders of sexual differentiation (DSD). Less frequent causes are growth hormone deficiency and idiopathic, functional abnormality of hypothalamus–pituitary–testicular axis without overt abnormality (4,5).

Since patients with micropenis are often dissatisfied with sexual quality of life and cosmetic appearances, psychological stigmatization is common (6,7). Thus, it was reported that earlier rather than later hormonal administration results in longer penile length (8,9). However, several concerns need to be addressed regarding early hormonal administration. As Husmann et al. (10) showed results in hypogonadotropic microphallic rats, early administration of testosterone caused increased pre-pubertal penile length. However, this eventually led to smaller phallic size than normal counterparts, suggesting that early administration leads to premature termination of pubertal phallic growth. This was associated with accelerating decrease of androgen

receptors (AR) before puberty consequent to administration of exogenous testosterone. Moreover, safety concerns about testicular germ cells and bone growth have not been addressed well.

Growth hormone deficiency causes micropenis even in the presence of intact hypothalamus-pituitary-gonadal axis (11). While the underlying molecular mechanism is unclear, the growth hormone/insulin-like growth factor-1 (IGF-1) axis possesses proactive effects for the action of testosterone and its potent derivative, dihydrotestosterone. Furthermore, it was reported to prevent the reduction of AR and to enhance growth in both penile fibroblast and testis, which could be beneficial in the treatment of micropenis (12). If above-mentioned facts still hold true in those with normal growth hormone/IGF-1 axis, we may be able to increase penile dimensions without concerns inherent to conventional androgen treatment.

In clinical settings, there are little evidence to prove effects of growth hormone on human micropenis. Growth hormone treatment in fifteen isolated growth hormone deficient boys showed significant improvement of penile length and testicular volume especially at the timing of prepubertal period (13).

Interestingly, recent study with adult patients who suffered from growth hormone deficiency proved that recombinant growth hormone improved dose-dependent relaxation of corpus cavernosum (14). The relaxing potency of human GH also increased in proportion to the elevation of level of cyclic guanosine monophosphate (cGMP).

Accordingly, the treatment of growth hormone may play an important role in the formation of penile structure and integrity of not only normal penis but that of micropenis. Also, growth hormone might have synergistic effect on activation of testosterone to prevent reduction of AR.

To address our hypothesis, we performed a study to test the effect of GH monotherapy or in combination with testosterone upon phallic dimensions, structural integrity and testicular size in microphallic rats.

II. Materials and Methods

2-1. Animals and experimental protocol

All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Seoul National University. Animal care complied with Association for Assessment and Accreditation of Laboratory Animal Care and National Institutes of Health guidelines. Seven timed-pregnant Sprague-Dawley rats were obtained one week before parturition. After parturition, pups remained with their mother until two weeks of age. Male pups were discriminated by females by longer anogenital length on the third postnatal day, although this was not always possible (Fig. 1). This explains the disparity in numbers among groups. Five study groups were evaluated for this investigation: normal controls (C), untreated microphallic rats (MP), microphallic rats treated by GH only (G), microphallic rats treated by testosterone (T) and microphallic rats treated by both GH and testosterone (GT).

Microphallus was induced in rat pups by weekly administration of leuprolide acetate (0.1 to 0.4mg/kg, dose escalation in every two weeks) from postpartum day five. On postpartum day 7, testosterone enanthate was weekly administered at a dosage of 0.4mg/kg/week. GH was administered subcutaneously at a dose of 2.5mg/kg in every other day during the treatment period. Following 14 weeks of treatment, rats were euthanized by aspiration of blood from the heart. Following anthropometric measurement of body weight and phallic dimensions, the penis was excised at the level of the crus and prepared for examination under stereomicroscopic control. For histochemistry, the area below penile os (distal area) that shows the typical bilobular shape of the corpus cavernosum was sliced and fixed in 4% formalin. For western blot,

the area near to the penile crus (proximal side) including more smooth muscle was resected and snap-frozen in liquid nitrogen and stored at -80°C.

2-2. Penile anthropometry

Body weight was measured every two weeks and at the time of necropsy. Stretched penile length was recorded by placing the phallus on stretching and measuring the length from the palpable proximal penile tip to the tip of the phallus using microcalipers.

2-3. Plasma testosterone and IGF-1

Blood was centrifuged and plasma was stored at -20°C until assay. Commercial ELISA kits were used for the assay of testosterone assay (Abcam, Cambridge, MA, USA) and IGF-1 (R&D systems, Minneapolis, MN, USA) was used. All assays were conducted in duplicates and the mean value was calculated and expressed. The sensitivity of the assay was 0.07 ng/ml and 4.4 pg/ml for the testosterone and IGF-1, respectively. The reported interassay coefficient of variation was 5% and 4.6% for each test.

2-4. Androgen receptor (AR) assay

Part of proximal penises were homogenized individually in a buffer consisting of 10 mM Tris, pH 7.4, 1.5 mM EDTA, 0.1% glycerol, 1mM 2-mercapto-ethanol, 10 mM Na molybdate. The homogenate was centrifuged at 150,000g for 30 minutes and fraction of cytosol were obtained. Equal aliquots (120 μg protein) of the penile cytosol fraction were run on 7.5% polyacrylamide SDS gels, and the proteins were transferred to PVDF

membrane (0.45um, GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) in 100V for 50 minutes, blocked with 5% BSA in PBS solution for 1 hour, and incubated with primary rabbit polyclonal antibody against a 110 kDa protein from HepG2 nuclear region in rat AR. Membranes were briefly rinsed with TBS-T buffer and incubated with secondary antibody conjugated with horseradish peroxidase (1:3000) for 60 minutes. Bands were detected by enhanced chemiluminescence (GE Healthcare Bio-Sciences). The films were scanned and each band density evaluated by densitometric analysis.

2-5. Histomorphometry

Fixed specimens were processed and embedded in paraffin wax followed by sectioning and staining with hematoxylin and eosin. Slides were de-paraffinized by three washes in xylene and rehydrated through a series of graded alcohol steps (100%, 95%, and 70%) and water, each for 5 min. Unless otherwise stated, all washes were performed three times in phosphate-buffered saline containing 0.05% Tween (pH 7.4) for 5 min each, and all incubations were carried out in a humid chamber at room temperature. Antigen retrieval was achieved by heating the slides in a microwave oven in a 0.01 M sodium citrate solution (pH 6.0) and subsequently cooling them for 30 min, followed by washing. Endogenous peroxidase activity was blocked by incubating the slides for 30 min in 1% hydrogen peroxide in methanol. Nonspecific binding was blocked by incubating the slides for 1 h with normal horse serum (Vector Laboratories, Inc., Burlingame, CA, USA). Slides were incubated with an anti-ACTA2 primary antibody (Santa Cruz Biotechnology, Dallas, Tx, USA), collagen Ⅰ and Ⅲ (all from Abcam) for 1.5 h at room temperature. After another wash, avidin-biotin-HRP complex (Vectastain Elite ABC kit, Vector Labs) was prepared and added according to the manufacturer's

instructions. 3,3 0 -Diaminobenzidine was used as the chromogen substrate, and photomicrographs were taken using an Olympus BX73 microscope (Olympus, Center Valley, PA) under bright field illumination.

Calculations of relative expression for SMA, collagen Ⅰ and Ⅲ, the number of fat globules and sinusoid density were carried out from photomicrograph, using Image Pro Plus software (version 4.5.0.29z, Media Cybernetics, Rockville, USA) was used. For each animal, assessment of above-mentioned variables was done from two different areas in the corpus cavernosum, which were examined under X100 magnification. Areas involved with SMA or collagens were calculated using histogram tool after a color segmentation of the image, based on automatic counting of the percentage of the pixels with the same color (brown for SMA and deep blue for collagen) as described previously (15). Likewise, sinusoidal density was calculated by dividing the sum of sinusoids by total areas consisting of trabecular network. The number of fat globules was directly counted and averaged.

2-6. Statistical Analysis

Data were expressed by mean ± SD. Data were analyzed using GraphPad Prism version 5 (Graph Pad Software Inc., San Diego, CA, USA) and one- way analysis of variance (ANOVA) followed by the Bonferroni post-test.

III. Results

Experimental overview, anthropometry and plasma level of testosterone and IGF-1

Usually a pregnant female rat gave birth to eight to 14 pups at a time. Three to 11 were found to be male and allocated to each group. Thirty-seven rats were included in the study and distributed into the five treatment conditions. Six rats went into C, eight to MP, nine to G, six to T and eight to GT. The mean body and testicular weights and mean plasma levels of testosterone and IGF-1 are described in Table 1. At the end of treatment, the mean body weight of all experimental groups was comparable. Secondary hypogonadism caused significant reduction of testicular volumes and testosterone treatment further aggravated these. Treatment of GH ameliorated significantly but partially ameliorated these. Compared to C, MP showed significantly lower mean plasma level of testosterone. Treatment with either testosterone or GH resulted in corresponding increase in plasma levels. Interestingly, plasma testosterone in G was slightly but significantly higher than MP. Plasma IGF-1 in those who received testosterone decreased compared to those who did not. Hence, the mean IGF-1 concentration in GT was not significantly different from C despite the GH supplementation.

Table 1. Comparison of mean body weight, mean testicular weight, mean plasma testosterone and mean IGF-1 among experimental groups.

	C	MP	G	T	GT
Body weight (g, SD)	588 (34)	579 (28)	575 (39)	552 (44)	567 (45)
Testis volume (ml, SD)	1.36 (0.11)	0.97 (0.05)$^{\#}$	1.26 (0.08)*	0.83 (0.06)$^{*\#}$	1.10 (0.09)$^{*\#}$
Plasma testosterone (ng/ml, SD)	3.72 (1.91)*	0.44 (0.05)$^{\#}$	2.08 (0.51)*	>25$^{*\#}$	>25$^{*\#}$
Plasma IGF-1 (ng/ml, SD)	1445 (155)	1432 (232)	1704(116)$^{*\#}$	628 (206)$^{*\#}$	1197 (126)$^{*\#}$

* denotes statistical significance ($p<0.05$) compared to MP.

# denotes statistical significance ($p<0.05$) compared to C.

Penile anthropometry

Compared to C, the MP rats had significantly reduced mean phallic dimensions, resulting in microphallus (Fig. 2). G phallic dimensions did not differ significantly from those of MP. Phallic dimensions in the G group did not differ significantly with those in the MP group, and whereas those in the testosterone group were significantly improved, they were not restored to control dimensions. In contrast, combination treatments normalised phallic dimensions to C sizes. To assess bone growth, adjusted penile lengths were calculated as the ratio of penile and right tibial lengths. Differences in this

parameter were similar to those of penile lengths, indicating minimal effects of the present treatments on bone growth (Fig. 3).

Androgen receptor expression in penile tissue

Comparison of penile AR expression at 14 weeks of age revealed higher expression in MP compared to C (Fig. 4). Treatment of testosterone alone resulted in suppression of AR expression but treatment of GH resulted in increased expression of AR. This resulted in highest expression of AR in G and comparable expression to MP in GT.

Histomorphometry

Exposure to hypogonadal insult after birth led to several obvious histologic changes (Fig. 5). Compared to the control group, MP showed reduced expression of SMA and increased expression of collagen Ⅰ and Ⅲ (Fig. 6, Fig. 7). Both types of collagen accumulated densely in the entire trabeculae. Moreover, multiple fat globules were found around sinusoidal spaces and decreased sinusoidal densities were identified. Treatment with GH resulted in significantly reduced expression of collagen Ⅲ, but did not affect other histologic parameters. Treatment with testosterone significantly increased SMA expression and sinusoidal density (Fig. 8, Fig. 9). This was also associated with a remarkable decrease in fat globules (Fig. 10). The expressions of collagen Ⅰ and Ⅲ, however, were not influenced by the testosterone treatment alone.

All types of histologic variables were improved by the combination treatment. Comparable results to C were found in expression of SMA, the number of fat globules

and sinusoidal density, but partial improvement was seen in collagen Ⅲ while no improvement was seen in collagen Ⅰ (Fig. 9, Fig. 10).

IV. Discussion

The prevalence of micropenis has been reported in various studies. Epidemiological study reported 0.015 % of men suffered from micropenis in United States between 1997 and 2000 (16). Mazen et al. published the micropenis rate was 0.062 % in Egypt (17). The recent studies showed higher prevalence than before in micropenis from 0.347 to 0.66 % (18,19).

In this study, we induced microphallic rats using hypogonadotropic hypogonadism, the most common cause of micropenis. They developed significantly smaller phalli due to reduction of smooth muscle and cavernosal spaces. Increased amount of collagens and accumulation of fat globules were also seen. Treatment with growth hormone alone had no beneficial effect on phallic dimensions, but partial improvement of collagen Ⅲ. Treatment with testosterone alone led to partial improvement of phallic dimensions and several histologic features, but failed to normalize them. On the other hand, combination treatment fully normalized all measured penile dimensions. Histological assessment also revealed a combined beneficial effect of dual hormonal treatment, though both failed to prevent increased amount of collagen Ⅰ. Although all rats under hypogonadal insult had significantly decreased testicular volume, the further loss of testicular volume seen in testosterone monotherapy was ameliorated by the addition of growth hormone.

The most salient finding of our study was that addition of growth hormone augmented the tropic effect of testosterone. We confirmed that testosterone monotherapy was significantly but partially effective in enhancing penile dimensions in hypogonadal rats, supporting previous findings (20, 21). The lack of tropic effect in growth hormone monotherapy supports the previous finding that the tropic effects of

growth hormone could only be seen in the presence of growth hormone deficiency (22). When combined, however, significant tropic effects sufficient to normalize penile dimensions were observed. This normalization was results of supplementary effect of GH to testosterone. Given that treatment of GH led to enhanced AR expression, it is rational to assume that testosterone effect could be augmented by GH treatment by increasing AR expression, resulting in normalized phallic growth. This could explain the reason of suboptimal preclinical results associated with testosterone treatment for micropenis.

Another salient finding worth mentioning was abnormal structural integrity inherent to microphallus. Despite reports claiming no relationship between penile length and satisfaction of sexual activity (23) patients with micropenis reportedly have absent or unsatisfactory sexual intercourse for the reasons that are not clear (24). This may be attributed to psychological problems, but penile structural problems resultant from hypogonadism may be partly responsible for this problem. Indeed, Traish et al. (25) reported loss of smooth muscle and accumulation of adipocytes in hypogonadal rats and testosterone alone sufficiently addressed these problems. In addition to confirming above-mentioned findings, we newly found changes in collagen and sinusoidal density, suggesting that micropenis is not just a matter of small-sized penis but a problem of structural integrity. These were best addressed by combination treatment.

Although collagen is a major constituent of the extracellular matrix and an essential component of the penis, an abnormal increase of extracellular matrix could be harmful, and accumulation may hinder engorgement of blood in sinusoid, explaining reduction of sinusoidal density. This accumulation of collagen can be referred to penile fibrosis (20). Penile fibrosis due to abnormal collagen accumulation may be prohibitive for penile growth and a harbinger of microphallus, along with eliciting venogenic erectile dysfunction and potentially reduced sexual activity. Our findings highlighted that

expression of collagen Ⅲ could be alleviated by GH treatment, despite persistent expression of collagen I. This beneficial effect inherent to only GH supports its use for combination with testosterone as opposed to monotherapy in treating penile fibrosis and preserving structural integrity. Despite the lack of effect on penile dimension, no treatment was not helpful in reducing collagen Ⅰ. The potential implication of increased amount of collagen Ⅰ on penile function should be explored in subsequent study.

The adverse effect of neonatal exposure to hypogonadism on future corporal integrity also has been explored elsewhere. Okumu et al. (26) reported reduced expression of genes and proteins relevant to smooth muscle differentiation and tone following neonatal low androgen milieu. In sum, we believe that a more proactive approach to micropenis may be needed to preserve penile integrity as well as penile dimensions. Circumventing the side effects from prolonged androgen exposure may be alleviated by combination with GH.

Our experiment revealed that the testosterone and/or growth hormone treatment did not cause any additional harm to testicular function apart from original effect of hypogonadism. The MP testis volume showed about 30% shrinkage, but those who received growth hormone showed significantly larger testicular volume. Conversely, those who received only testosterone showed more shrinkage than MP. Though the reason for this tropic effect of growth hormone on testis has not been clarified, several clinical (27, 28) as well as preclinical reports (29, 30) suggested that administration of growth hormone could be helpful in promoting testicular growth. Our data supported these findings reporting that serum testosterone was increased by growth hormone treatment. Interestingly, testosterone treatment decreased serum IGF-1 assumed to diminish its effect. This could be another good reason that growth hormone should be incorporated in the treatment of micropenis, avoiding the adverse effect of testosterone monotherapy on testicular growth. Although, we did not measure bone growth, somatic

growth was not different from the control and treated rats. This would be indirect evidence for bone safety in this problem.

In the aspect of the relationship between androgen receptor and growth hormone, most studies were focused on prostate cancer growth. In the analysis of immature rat model, treatment of growth hormone induced increase of significantly steady-state mRNAs of androgen receptor in the prostate. Reiter et al. concluded growth hormone is involved in the regulation of synthesis of androgen receptor (31). Another study suggested induction of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) axis signaling has close relations with risk of prostatic cancer aggravation. In case of growth hormone receptor (GHR) knockout mice, retardation of prostatic tumorigenesis signaling was shown at disrupted growth hormone (32, 33). GH is suppressed by androgens in prostate cancer cells. Androgen receptor inhibitors causes increase in local growth hormone expression. Instead, excessive growth hormone induces androgen receptor splicing variants (ARv7) and IGF-1 expression in prostate cancer cells (34, 35, 36).

Though above studies are mainly about the relationship between growth hormone and androgen receptor in prostate cells, it may be speculated that the effect of testosterone repressed IGF-1 expression in testosterone only treated T group of our results. In our results of plasma IGF-1 in table 1, level of testosterone-only administered group (T) was significantly decreased. Also, growth hormone-only administered group (G) showed statistically significant increase in testosterone level. Theoretically, these phenomena may be explained by above mechanism that GH was suppressed by testosterone and prevented androgen receptor inactivation by inducing androgen receptor variants.

However, our study has some limitations to apply these results to clinical setting. Considering timing and period of administration of testosterone and growth hormone, there are some differences between our study and clinical practice. Although this study

were designed in a preventive manner, administration of testosterone is usually attempted at pre-pubertal period as a therapeutic manner. Testosterone enanthate was administered weekly from postpartum day 7 in our study. In case of growth hormone, it was administered in every other day. Nevertheless, our results still have clinical importance to prove the effect of growth hormone on micropenis.

It is still enigmatic when and how untoward histomorphological changes would occur in penile tissue. Also, it should be determined when and how to apply testosterone to prevent pathophysiological damage of penile structure. Furthermore, the effect of testosterone on the inactivation of androgen receptor according to dose escalation should be verified in high dose testosterone setting.

Consecutively, future study will be focused on pathophysiological changes of penile tissue regarding testosterone dose and various timings of administration.

V. Conclusions

Our experiment suggested that combination of testosterone with growth hormone could be effective strategy to circumvent the problems inherent to monotherapy. Growth hormone could be a valuable adjunct to testosterone monotherapy in both enhancing penile growth and preserving structural integrity. With regards to safety, the treatment appeared to less harmful for testicular growth than testosterone monotherapy.

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Figure legends

Fig. 1 Difference of ano-genital length between female and male pups. Red colored 'F' indicates female ano-genital distance and blue colored 'M' indicates male ano-genital distance.

Fig. 2 Comparison of stretched penile length and penile weight among five studied groups. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 3 Comparison of penile length / right tibial length ratios among five studied groups. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 4 Comparison of penile androgen receptor (AR) expression among five groups. Representative tracing and densitometry are seen in upper and lower panel, respectively. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 5 Immunohistochemical localizations of collagen Ⅰ, Ⅲ and smooth muscle actin were compared among five experimental groups. Relative amount of each expressions was semiquantitatively measured. Scale bar indicates 100um.

Fig. 6 Immunohistochemical localizations of collagen I was compared among five experimental groups. Relative amount of each expressions was semiquantitatively measured. Scale bar indicates 100um. Quantitative evaluation of relative expression was depicted in densitogram. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 7 Immunohistochemical localizations of collagen III was compared among five experimental groups. Relative amount of each expressions was semiquantitatively measured. Scale bar indicates 100um. Quantitative evaluation of relative expression was depicted in densitogram. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 8 Immunohistochemical localizations of smooth muscle actin was compared among five experimental groups. Relative amount of each expressions was semiquantitatively measured. Scale bar indicates 100um. Quantitative evaluation of relative expression was depicted in densitogram. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 9 Quantitative evaluation of relative expression was depicted in densitogram of sinusoidal density. The sinusoidal density was also measured and described. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 10 Quantitative evaluation of relative expression was depicted in densitogram of sinusoidal density and the number of fat globules. The number of fat globules was also measured and described. As C and MP serve as references for comparison, statistical comparison was done for either of these variables.

* denotes statistically significant difference ($p<0.05$) compared to MP.

# denotes statistically significant difference ($p<0.05$) compared to C.

Fig. 1

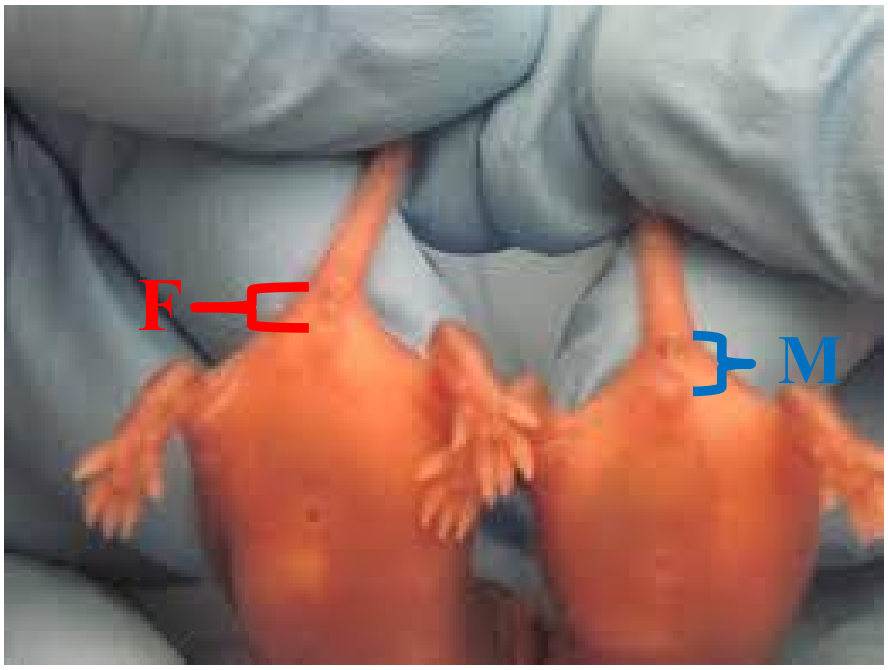


Fig. 2

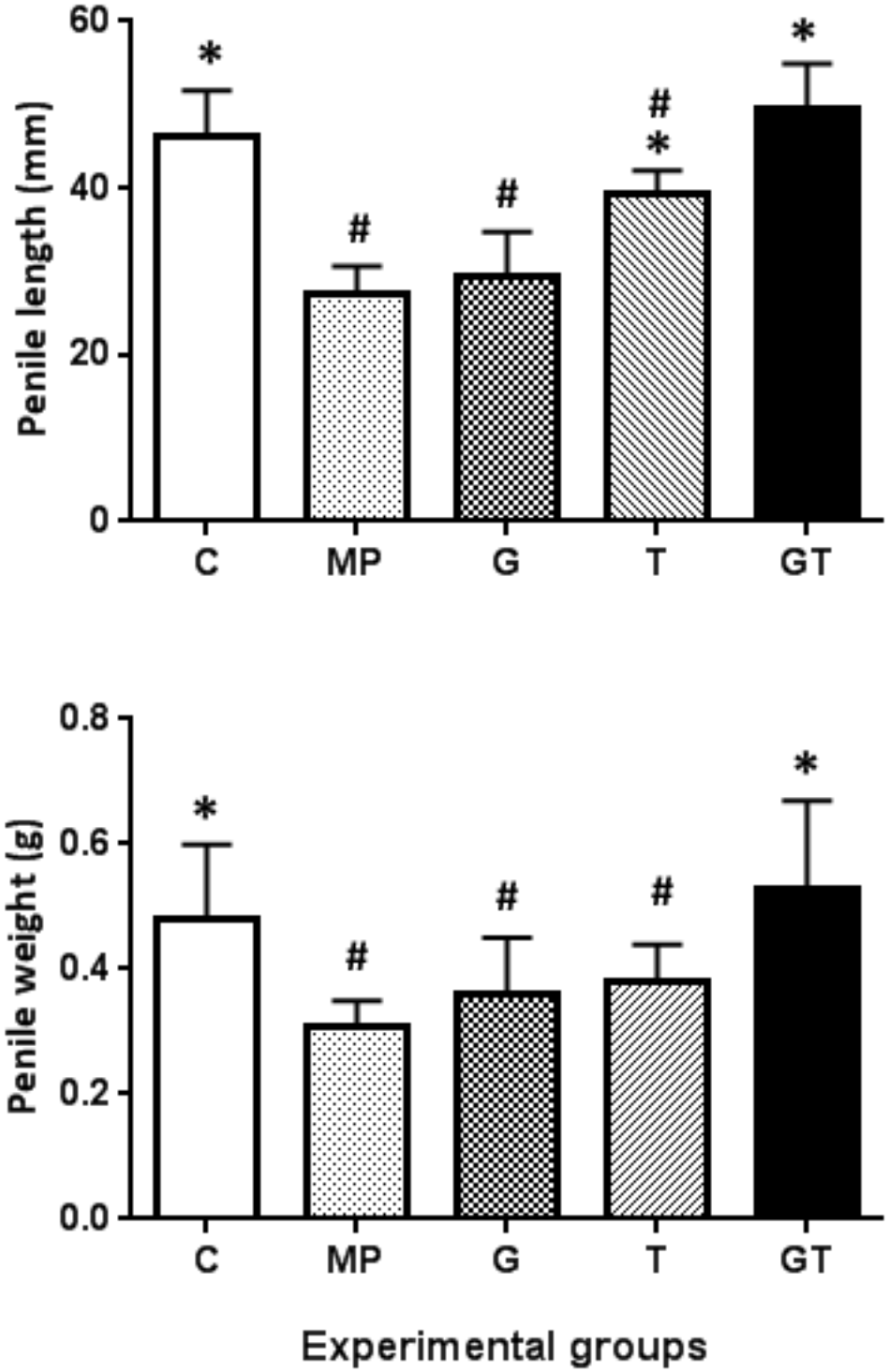

Penile length (mm)
60
40
20
0
*
#
#
#
*
*
C
MP
G
T
GT
Penile weight (g)
0.8
0.6
0.4
0.2
0.0
*
#
#
#
*
C
MP
G
T
GT
Experimental groups

Fig. 3

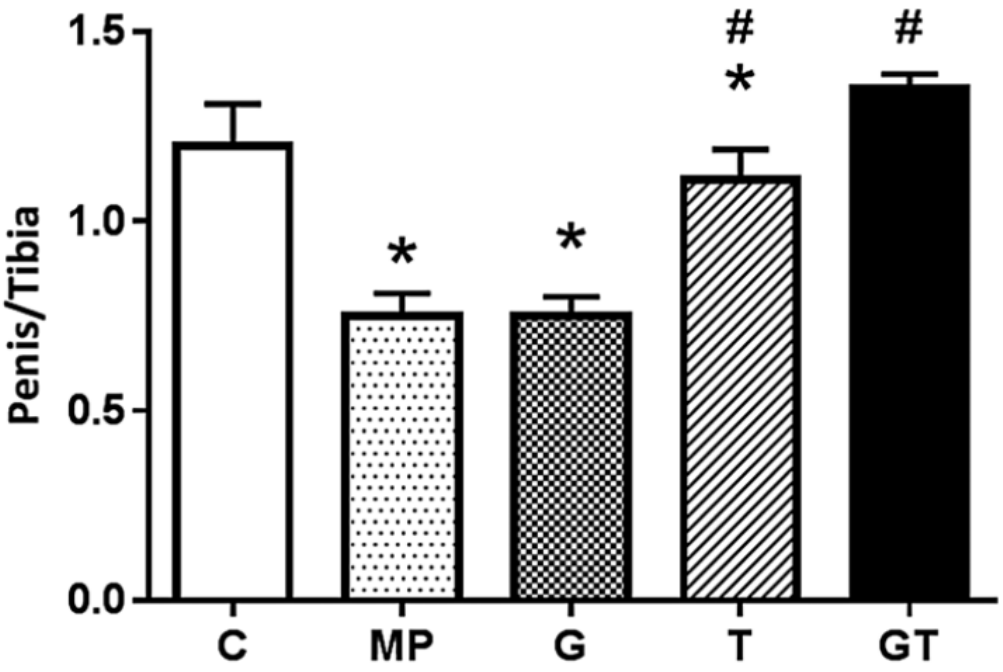


Fig. 4

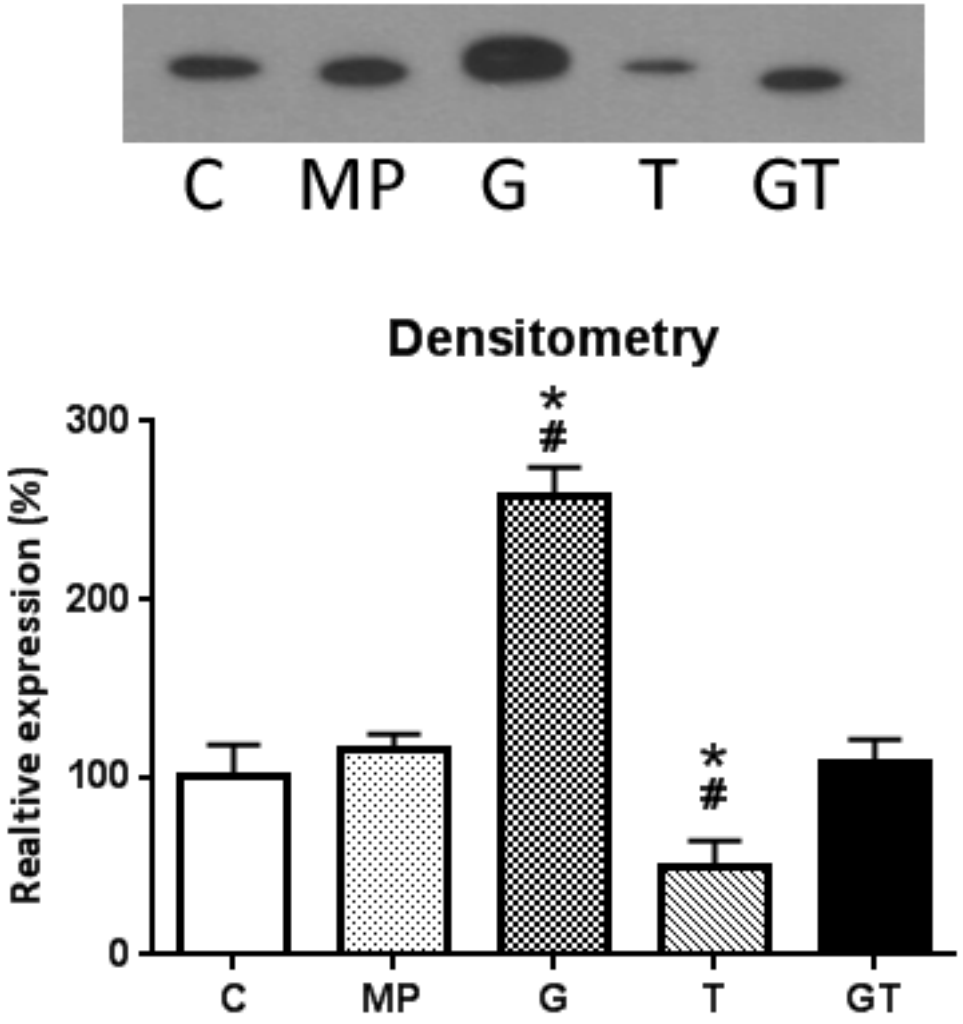

C
MP
G
T
GT
Densitometry
Realtive expression (%)
300
200
100
0
*
#
*
#
C
MP
G
T
GT

Fig. 5

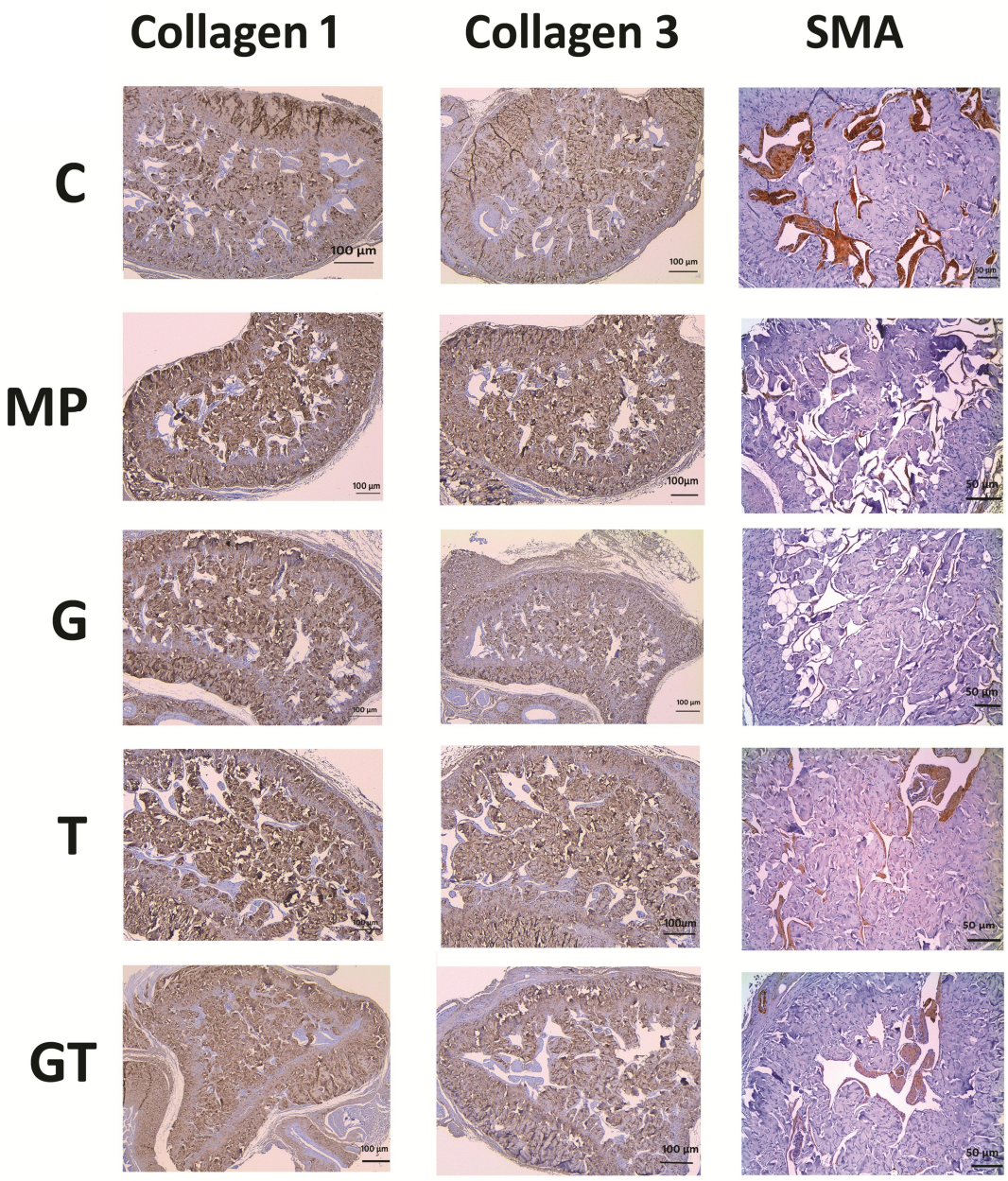
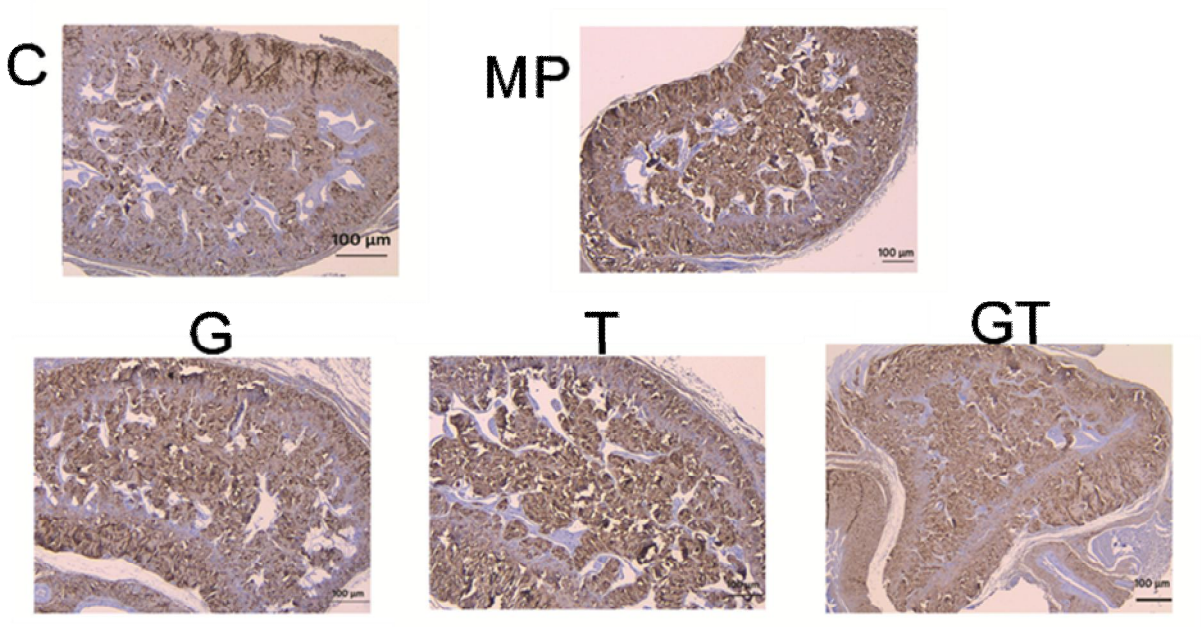


Fig. 6



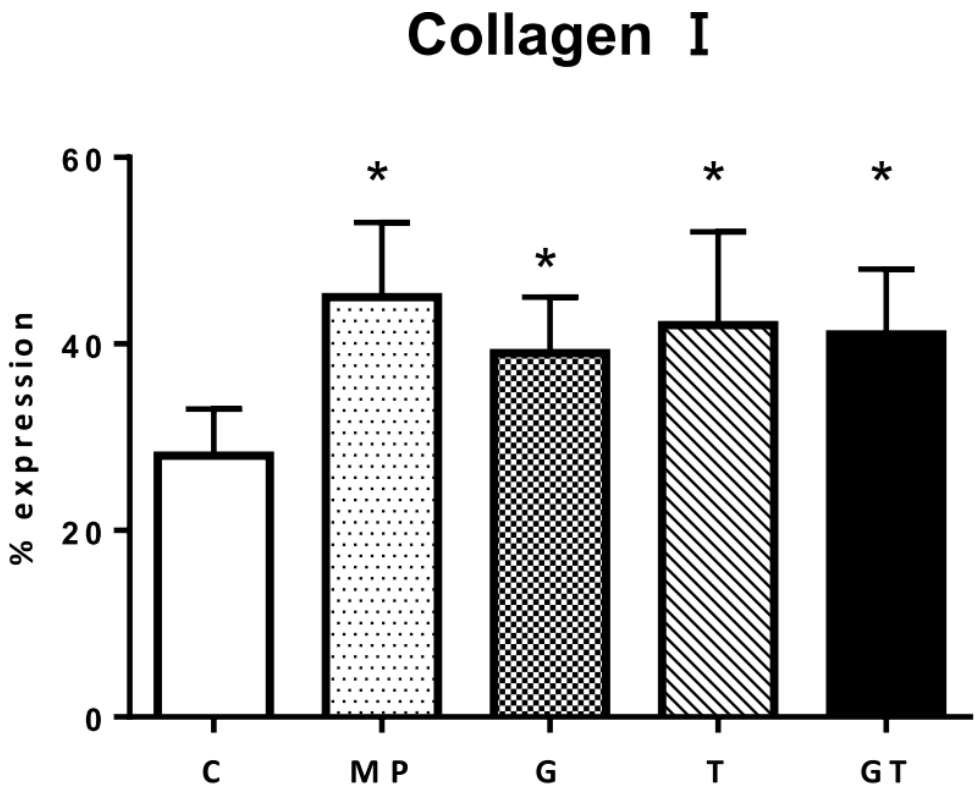
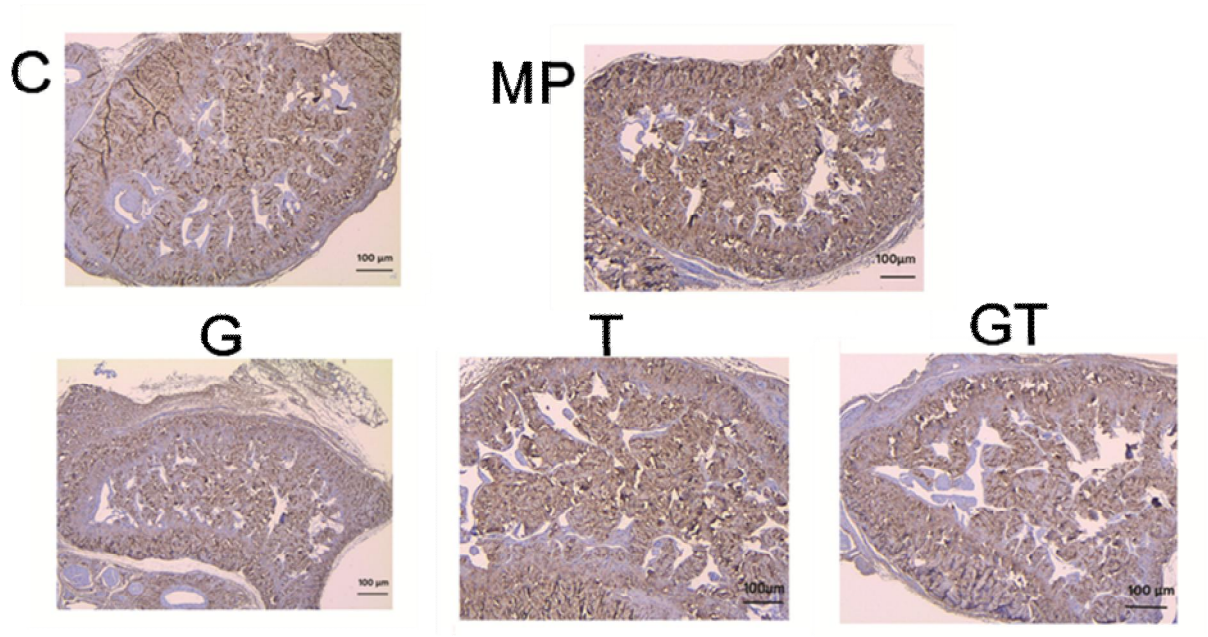


Fig. 7


C
MP
G
T
GT
100 µm
100µm

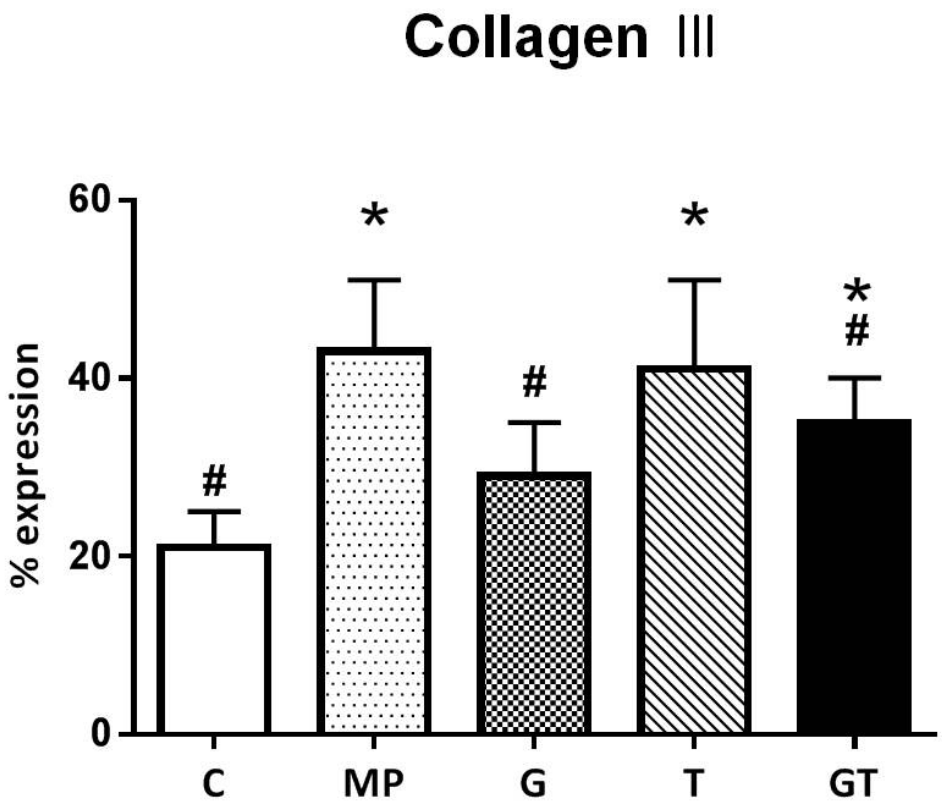
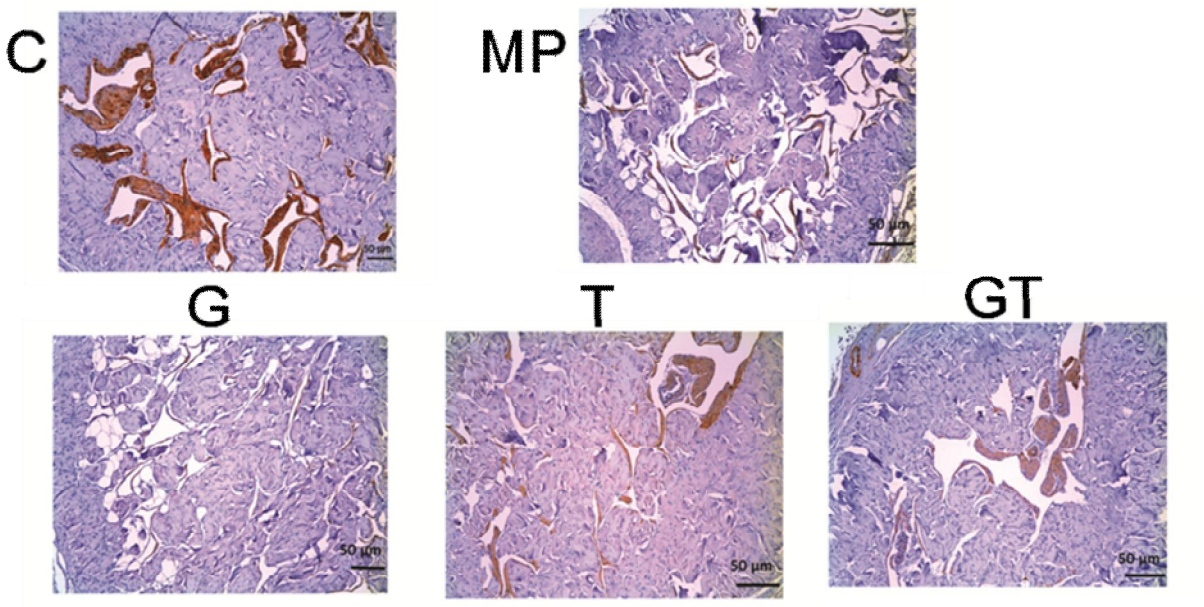

Collagen III
% expression
60
40
20
0
*
*
*
#
#
#
C
MP
G
T
GT

Fig. 8



Smooth Muscle Actin

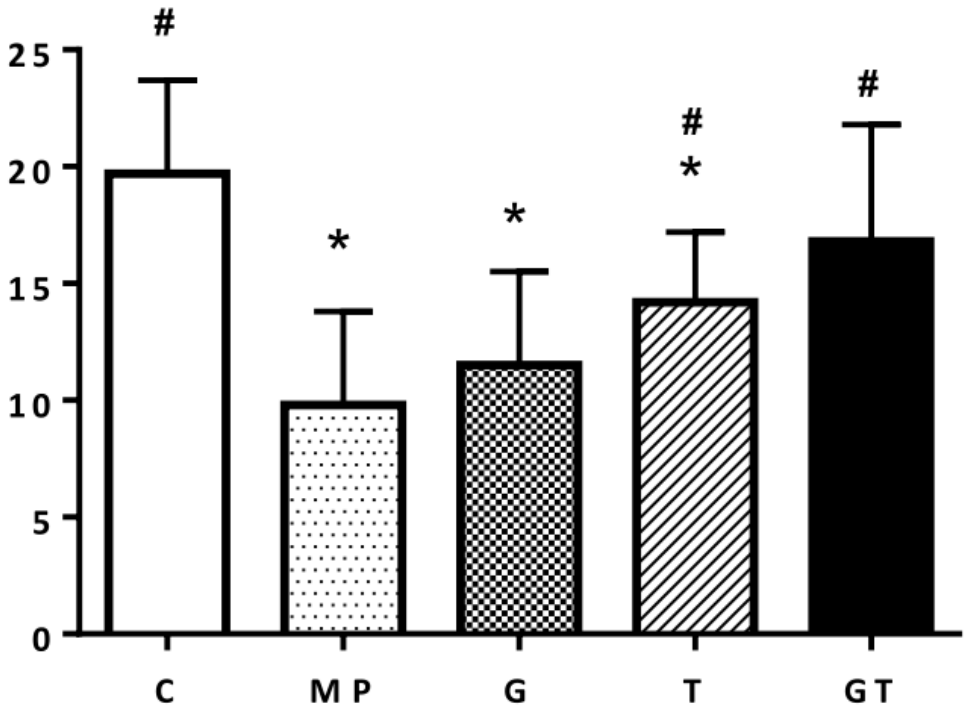


Fig. 9

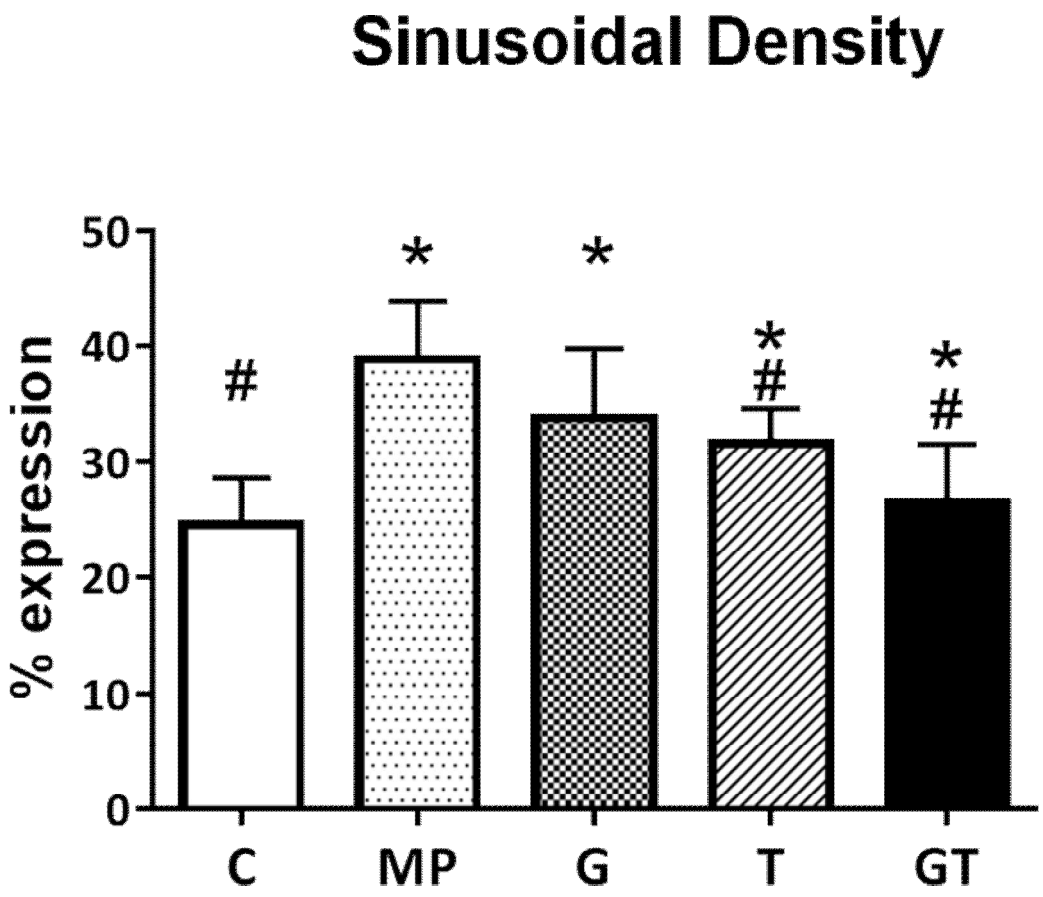
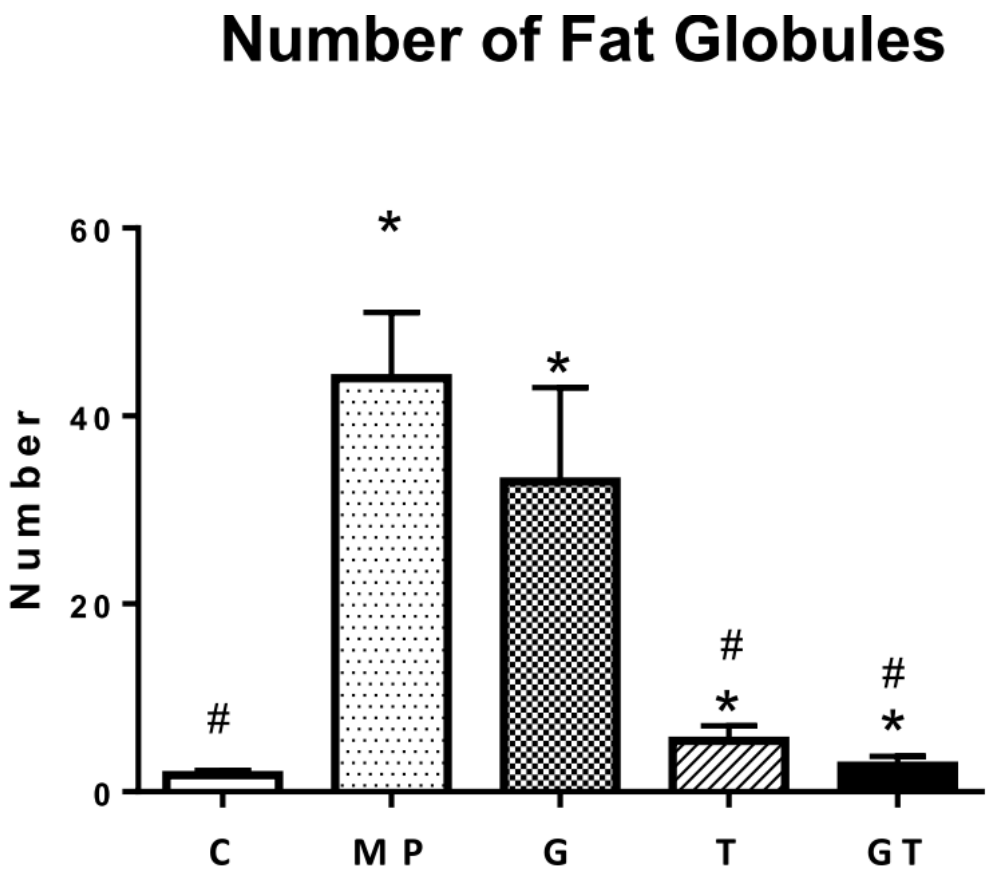


Fig. 10



Endocrine CONNECTIONS | J K Oh et al. | Growth hormone as cotreatment for micropenis | 7:11 | 1150–1157

RESEARCH

Effects of combined growth hormone and testosterone treatments in a rat model of micropenis

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Abstract

Although it is well known that penile growth is dependent on androgens, few clinical studies have reported successful treatment of micropenis with testosterone, likely due to concerns regarding the efficacy and safety of prolonged testosterone use. Thus, we assessed the synergenic effects of growth hormone (GH) treatments with and without testosterone on phallic growth in a rat model of micropenis. Fifty Sprague–Dawley rats were assigned to control (C), microphallus (MP), testosterone, GH (G) and GH plus testosterone (GT) treatment groups, and microphallus was induced by secondary hypogonadism. Pre-pubertal treatments with testosterone, GH or the combination were initiated from 7 days after birth and were maintained until 12 weeks of age. To assess the efficacy of treatments, phallic dimensions were determined and histological markers of cavernosal integrity were evaluated. Skeletal and gonadal safety profiles of the treatments were then assessed according to right tibial lengths and testicular weights, respectively. No monotreatments normalised penile dimensions, whereas combination treatments led to complete restoration. The combination treatment also prevented decreases in histological indicators of cavernosal integrity, including smooth muscle actin and collagen III expression levels and fat globule accumulation and sinusoidal density. These synergenic effects of GH treatments on penile growth may follow changes in androgen receptor expression levels and were accompanied by decreased testicular volume losses. Although the physiological conditions of phallic growth differ between humans and rats, this proof-of-concept study provides a strategy for circumventing the problems of testosterone monotherapy for human micropenis.

Key Words
- micropenis
- testosterone
- growth hormone
- combination treatment

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Introduction

The term micropenis encompasses a range of congenital and acquired conditions that present as abnormally short penises with a stretched length of more than 2.5 standard deviations (S.D.) below the mean for a particular age, albeit with no hypospadias, as indicated by normal morphology of the urethral meatus at the tip of the glans penis. Micropenis is commonly associated with testosterone deficiencies from 12 weeks of gestation, with normal placental human chorionic gonadotropin-induced testosterone levels during organogenesis (1). Among patients with hypogonadotropic hypogonadism, functional deficiencies are more frequently associated with foetal luteinising hormone than with disorders of sexual differentiation. Less frequent causes include growth hormone (GH) deficiencies and idiopathic functional abnormalities of the hypothalamic–pituitary–testicular

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axis, and overt abnormalities are generally absent under these conditions (2, 3).

Patients with micropenis are often dissatisfied with their sexual quality of life and cosmetic appearance and stigmatisation is common (4, 5). Hence, hormone treatments have been considered, and these reportedly result in longer penile lengths when administered early (6, 7), albeit with concerning side effects. In a study of hypogonadotropic microphallic rats by Husmann *et al.* (8), testosterone treatments increased pre-pubertal penile lengths but resulted in smaller phallic sizes than in normal rats, suggesting that early administration leads to premature termination of pubertal phallic growth. These observations were associated decreased androgen receptor (AR) expression levels before puberty, clearly due to the presence of exogenous testosterone. Moreover, effects on the growth of testicular germ cells and bone may warrant safety concerns, and these remain insufficiently characterised.

GH deficiency is the primary cause of micropenis, even in the presence of intact hypothalamic–pituitary–gonadal axis (9). Although the underlying molecular mechanisms have not been described, the GH–insulin-like growth factor 1 (IGF1) axis is a proactive mediator of the actions of testosterone and its potent derivative dihydrotestosterone. Accordingly, stimulation of IGF1 signalling prevented reductions of AR expression and enhanced penile fibroblast proliferation and testis growth, indicating a target for the treatment of micropenis (10). Hence, under conditions of normal GH and IGF1 activities, penile dimensions may be increased without the side effects of conventional androgen treatments.

Herein, we characterised the effects of GH monotherapy and GH–testosterone combination therapy on phallic dimensions, structural integrity, bone growth and testicular size in microphallic rats.

Materials and methods

Animals and experimental protocol

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Seoul National University. Animal husbandry was performed in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care and the National Institutes of Health guidelines. Seven timed-pregnant Sprague–Dawley rats were obtained at 1 week before parturition, and after parturition, pups were raised with their mothers until 2 weeks of age. Male pups were distinguished from females by longer anogenital lengths on the third postnatal day, although this distinction was not always clear, leading to differing numbers of pups between study groups. Pups were assigned to the following treatment groups: normal control (C), untreated microphallic rats (MP), microphallic rats treated with GH only (G), microphallic rats treated with testosterone and microphallic rats treated with both GH and testosterone (GT).

Microphallus was induced in rat pups by weekly administration of leuprolide acetate (74381536, intramuscular administration of 5 µg/kg in 0.1 mL saline solution, Sigma-Aldrich) at 0.1–0.4 mg/kg, with biweekly dose increases from postpartum day 5. From postpartum day 7, testosterone enanthate (Savient Pharmaceuticals, East Brunswick, NJ, USA) was administered intramuscularly at 0.4 mg/kg/week. GH (recombinant human growth hormone bulk solution, LG Life Sciences, Korea) treatments were administered subcutaneously on alternate days at 2.5 mg/kg, and after 14 weeks, rats were killed by aspiration of blood from the heart. Following anthropometric measurements of body weights and phallic dimensions, penises were excised at the level of the crus and were examined under stereomicroscopic control. To evaluate the effects of treatments on bone growth, right tibia lengths were measured using a digital calliper with an accuracy of 0.01 mm (Merox, Vienna, Austria). The area below the penile os (distal area) that shows the typical bilobular shape of the corpus cavernosum was isolated, sliced and fixed in 4% formalin for immunohistochemical analyses. Western blotting analyses were performed using tissue samples from the area near the penile crus (proximal side), which includes smooth muscle. These samples were resected, snap-frozen in liquid nitrogen and stored at −80°C.

Penile anthropometry

Body weights were measured every 2 weeks and at the time of necropsy. Stretched penile lengths were recorded by stretching the phallus and measuring the length from the palpable proximal penile tip to the tip of the phallus using micro-callipers. Adjusted penile lengths were recorded as ratios of penile and tibial lengths.

Plasma testosterone and IGF1 assays

Aliquots of blood were centrifuged, and the resulting plasma samples were stored at −20°C. After thawing

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plasma, assays of testosterone and IGF1 were performed using commercial ELISA kits (Abcam and R&D Systems, respectively). All assays were conducted in duplicate, and mean values were calculated. Assays were sensitive to 0.07 ng/mL testosterone and 4.4 pg/mL IGF1, with reported inter-assay coefficients of variation of 5 and 4.6%, respectively.

AR assays

Proximal parts of penises were homogenised individually in a buffer containing 10 mM Tris (pH 7.4), 1.5 mM EDTA, 0.1% glycerol, 1 mM 2-mercapto-ethanol and 10 mM Na molybdate. Homogenates were centrifuged at 150,000*g* for 30 min, and cytosolic fractions were collected. Aliquots of the penile cytosol fractions containing 120 µg of protein were run on 7.5% polyacrylamide (PAGE) gels in sodium dodecyl sulphate, and the proteins were then transferred to PVDF membranes (0.45 µm, GE Healthcare Bio-Sciences) at 100 V for 50 min. Membranes were blocked with 5% bovine serum albumin in PBS solution for 1 h and were then incubated with primary rabbit polyclonal antibody against a 110 kDa rat AR protein from the HepG2 nuclear region (1:1000, ab133273, Abcam). Subsequently, membranes were rinsed briefly with TBS-T buffer and were incubated with secondary horseradish peroxidase-conjugated antibody (1:3000) for 60 min. Bands were detected using enhanced chemiluminescence (GE Healthcare Bio-Sciences), and exposed films were scanned for densitometric analyses of band densities.

Histomorphometry

Fixed specimens were processed and embedded in paraffin wax and were then sectioned and stained with haematoxylin and eosin. Slides were de-paraffinised using three washes with xylene and were then rehydrated by incubating in a graded series of alcohol (100, 95 and 70%) and water solutions for 5 min. Unless otherwise stated, all washes were performed three times in PBS containing 0.05% Tween (pH 7.4) for 5 min each, and all incubation steps were conducted in a humid chamber at room temperature. Antigen retrieval was achieved by heating slides in a microwave oven in 0.01 M sodium citrate (pH 6.0), cooling for 30 min and then washing 3 times in PBS containing 0.05% Tween. Endogenous peroxidase activity was blocked using 1% hydrogen peroxide in methanol for 30 min, and non-specific binding was blocked by incubating in normal horse serum for 1 h (Vector Laboratories, Inc.). Slides were incubated with an anti-ACTA2 primary antibody (1:1000, sc-130617, Santa Cruz Biotechnology) and collagen types I (1:1000, ab34710, Abcam) and III (1:1000, ab7778, Abcam) for 1.5 h at room temperature. After washing, an avidin–biotin–HRP complex (VECTASTAIN Elite ABC kit, Vector Labs) was prepared and added according to the manufacturer's instructions. Finally, 3,3,0-diaminobenzidine was used as the chromogen substrate and photomicrographs were taken using an Olympus BX73 microscope (Olympus) under bright field illumination.

Relative expression levels of smooth muscle actin (SMA) and collagen types I and III were determined, and numbers of fat globules and sinusoid densities were calculated from photomicrographs taken at 100× magnification using Image Pro Plus software (version 4.5.0.29z, Media Cybernetics, Rockville, MD, USA). All variables were assessed in two different areas of the corpus cavernosum for each animal. Areas with SMA or collagen expression were calculated from percentages of pixels with the same colour (brown for SMA and deep blue for collagen) using a histogram tool after colour segmentation of the image as described previously (11). Similarly, sinusoidal densities were calculated by dividing the sum of sinusoids by the total area of the trabecular network. Numbers of fat globules were counted and averaged directly.

Statistical analysis

Data are expressed as means ± S.D. Data were analysed using GraphPad Prism version 6 (Graph Pad Software Inc.), and differences were identified using one-way ANOVA followed by the Bonferroni *post hoc* test.

Results

Experimental overview, anthropometry and plasma testosterone and IGF1 levels

Pregnant female rats predominantly gave birth to 8–14 pups at a time, and 3–11 of these were males and were allocated to treatment groups. A total of 37 male rats were allocated to groups C, MP, G, testosterone and GT (6, 8, 9, 6 and 8 rats, respectively). Anthropometric assessments of mean body weights, right tibia lengths and testicular weights are presented with plasma testosterone and IGF1 levels in Table 1. After treatments, mean body weights and tibia lengths were comparable between all experimental groups. In contrast, leuprolide pretreatment caused

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Table 1 Comparison of mean body weight, mean testicular weight, mean plasma testosterone and mean IGF-1 among experimental groups.

	C	MP	G	Testosterone	GT
Body weight (g, S.D.)	588 (34)	579 (28)	575 (39)	552 (44)	567 (45)
Testis volume (mL, S.D.)	1.36 (0.11)	0.97 (0.05)[b]	1.26 (0.08)[a]	0.83 (0.06)[ab]	1.10 (0.09)[a,b]
Tibial length (mm)	38.4 (2.6)	36.6 (3.1)	39.1 (2.2)	35.5 (3.3)	36.8 (2.9)
Plasma testosterone (ng/mL, S.D.)	3.72 (1.91)[a]	0.44 (0.05)[b]	2.08 (0.51)[a]	27.5[a,b]	29.2[a,b]
Plasma IGF-1 (ng/mL, S.D.)	1445 (155)	1432 (232)	1704 (116)[a,b]	628 (206)[a,b]	1197 (126)[a,b]

[a]Denotes statistical significance ($P<0.05$) compared to MP; [b]denotes statistical significance ($P<0.05$) compared to C.

secondary hypogonadism, with significant reductions in testicle volumes, and these conditions were further aggravated by testosterone treatments in MP and G groups. Treatments with GH led to significantly larger testis volume and higher plasma testosterone level, ameliorating changes associated with hypogonadism, but did not correct the problem completely. Compared with rats in the C group, those in the MP group had significantly lower mean plasma testosterone levels. Treatments with testosterone and GH resulted in corresponding increases in plasma levels of these agents, whereas plasma testosterone levels were slightly but significantly higher in the G group than in the MP group. Plasma IGF1 levels were lower in testosterone-treated rats that in untreated rats, and mean plasma IGF1 concentrations in the GT group did not differ significantly with those in the C group, despite GH supplementation.

Penile anthropometry

Comparisons of phallic lengths and girths are presented in Fig. 1. Microphallus was confirmed in rats of the MP group, with significantly smaller mean phallic dimensions than in the C group. Phallic dimensions in the G group did not differ significantly with those in the MP group, and whereas those in the testosterone group were significantly improved, they were not restored to control dimensions. In contrast, combination treatments normalised phallic dimensions to C sizes. To assess bone growth, adjusted penile lengths were calculated as the ratio of penile and right tibial lengths. Differences in this parameter were similar to those of penile lengths, indicating minimal effects of the present treatments on bone growth.

Androgen receptor expression in penile tissues

Comparisons of penile (Fig. 2) AR expression levels in 14-week-old rats revealed higher expression in the MP group than that in the C group. Treatments with testosterone alone suppressed AR expression, whereas treatments with GH resulted in increased expression of AR. The highest AR expression levels were observed in the G group, and these were comparable MP and GT groups.

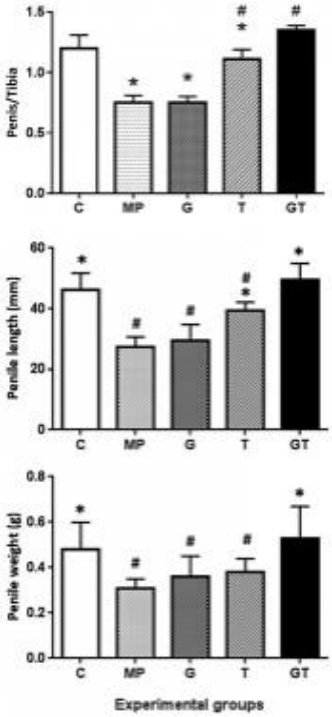

Figure 1
Comparisons of stretched penile lengths, penile weights and penile length/right tibial length ratios among the five study groups; comparisons of treatments are made with control (C) and microphallus (MP) groups; *$P<0.05$ compared with the MP group; #$P<0.05$ compared with the C group.

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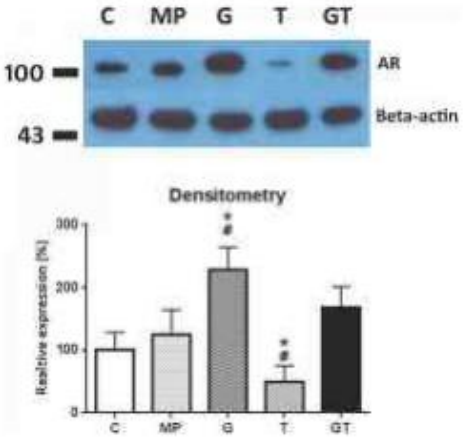

Figure 2
Comparisons of penile androgen receptor (AR) expression between treatment groups; representative traces and corresponding densitometric analyses are presented in upper and lower panels, respectively. Comparisons were made with C and MP groups; $^{*}P<0.05$ compared with the MP group; $^{\#}P<0.05$ compared with the C group.

Histomorphometry

Exposures to hypogonadal insults after birth led to several obvious histologic changes (Fig. 3A and B). Compared with rats of the C group, MP rats had reduced SMA expression control and increased collagen I and III expression levels. Both collagen types accumulated densely throughout the trabeculae. Moreover, multiple fat globules were found around sinusoidal spaces, and decreased sinusoidal densities were identified. Treatments with GH significantly reduced the expression of collagen III, but did not affect other histologic parameters. Moreover, testosterone treatments significantly increased SMA expression levels and sinusoidal densities and led to marked decreases in fat globule numbers. Expression levels of collagens I and III, however, were not influenced by testosterone treatments alone.

All histologic parameters were improved by combination therapy, with comparable SMA expression levels, numbers of fat globules and sinusoidal densities as those in the C group. But collagen III levels were only partially restored, and no changes in collagen I levels were identified.

Discussion

After inducing hypogonadotropic hypogonadism in microphallic rats, significantly smaller phalli were accompanied by reductions in smooth muscle and cavernosal spaces and increased collagen levels and fat globule accumulations. Treatments with GH alone did not affect phallic dimensions, and only partial improvements of collagen III levels were observed under these conditions. Treatments with testosterone alone led to improvements in phallic dimensions and other histologic features, but failed to restore these features to controlled levels. In contrast, combination treatments completely normalised all penile dimensions. Histological assessments showed corresponding benefits of combined hormone treatments, and although collagen I overexpression was not prevented, all treated microphallic rats had significantly decreased testicular volumes, and further losses of testicular volumes due to testosterone monotherapy were ameliorated.

The salient observation of this study was that cotreatments with GH augment the tropic effects of testosterone. We confirmed that testosterone monotherapy significantly, albeit partially, restores penile dimensions in hypogonadal rats, as shown previously (12, 13). Our data also corroborate previous observations of the limited tropic effects of GH monotherapy in the absence of GH deficiencies (14). In contrast, the tropic effects of combined treatments with GH and testosterone were sufficient to normalise penile dimensions. Because GH treatments enhanced AR expression, we suggest that the effects of cotreatments with testosterone are mediated by this receptor and that the ensuing signalling normalises phallic growth. Hence, the suboptimal preclinical results of testosterone treatments may reflect insufficient AR expression.

We observed abnormal structural integrity under the present conditions of microphallus. Previous reports show no relationship between penile lengths and sexual satisfaction (15), whereas other studies indicate that patients with micropenis have unsatisfactory or no sexual intercourse for reasons that were not specified (4). Taken together, these studies suggest that although psychological factors likely contribute to the sexual insufficiencies of hypogonadism, penile structural features are at least partly responsible. In accordance, Traish *et al.* (16) reported loss of smooth muscle and accumulation of adipocytes in hypogonadal rats, and these pathological traits were adequately addressed by testosterone treatments alone. In addition to confirming these observations, we found changes in collagen expression and sinusoidal densities, suggesting that micropenis is compounded by structural integrity and that combination treatments with testosterone and GH restore penis sizes and structural integrity.

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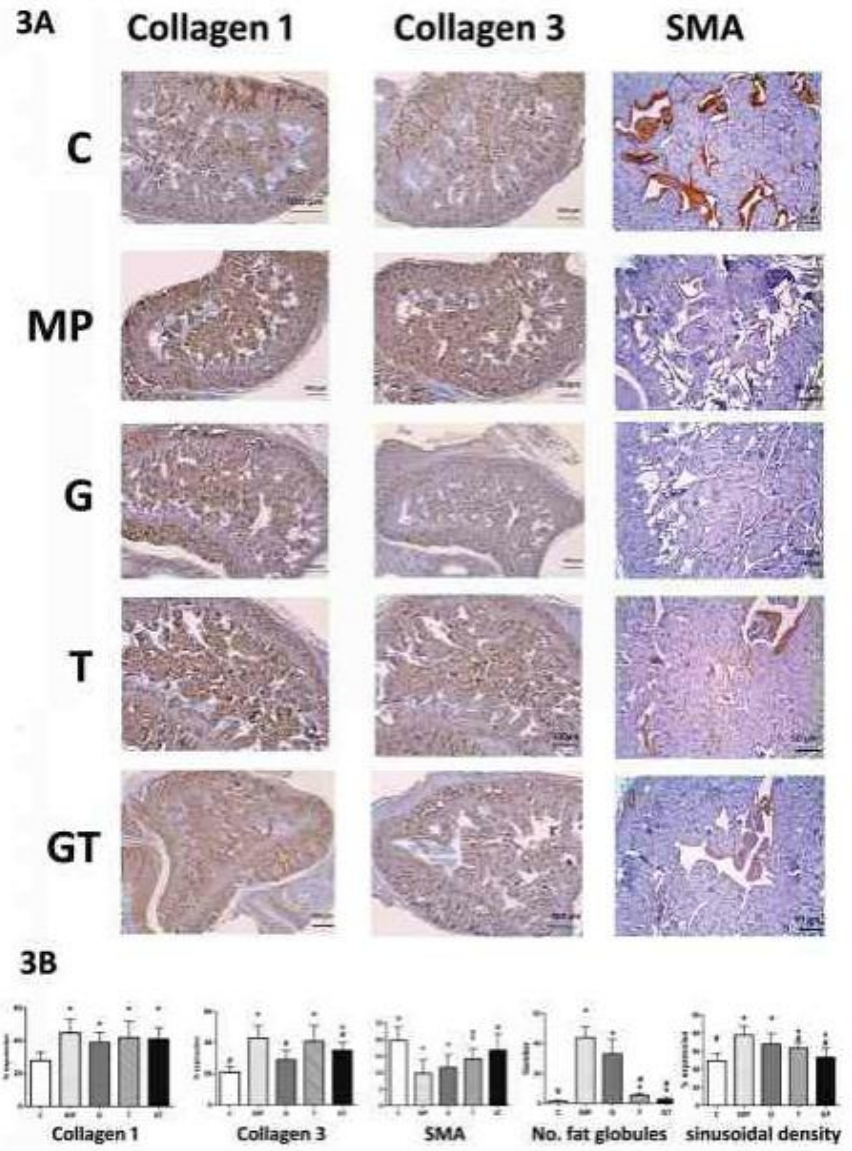

Figure 3
(A) Immunohistochemical localisation of collagens I and III and smooth muscle actin; relative expression levels were determined semiquantitatively; scale bar, 100 µm. (B) Relative expression levels of collagens I and III and sinusoidal densities and the numbers of fat globules; expression data are presented in the densitogram. Comparisons were made with C and MP groups; $^{*}P<0.05$, compared with the MP group; $^{\$}P<0.05$ compared with the C group.

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Although collagen is a major constituent of extracellular matrixes and is an essential component of the penis, excessive extracellular matrix is undesirable and collagen accumulation may hinder sinusoidal engorgement, leading to reduced sinusoidal densities. Collagen accumulation in patients with hypogonadism is often referred to as penile fibrosis (17) and may prohibit penile growth and contribute to venogenic erectile dysfunction and potentially reduced sexual activity. In our experiments, collagen III overexpression was alleviated by GH treatments. Although collagen I expression persisted under these conditions, these benefits are exclusive to GH treatments, supporting its use in combination with testosterone to limit penile fibrosis and preserve structural integrity. Although the implications of increased collagen III levels on penile function require further study, neither of the present monotreatments were ineffective at reducing collagen III levels, despite failing to affect penile dimensions.

Adverse effects of neonatal hypogonadism on subsequent corporal integrity were previously investigated by Okumu *et al.* (17). In their study, neonatal androgen deficiencies were associated with reduced expression levels of genes and proteins that play roles in smooth muscle differentiation and tone. Taken with these studies, our data indicate that proactive treatments of micropenis may successfully preserve penile integrity and penile dimensions. Specifically, the side effects of prolonged androgen exposure may be alleviated by cotreatments with GH.

We found that GH treatments alone or in combination prevented loss of testicular function beyond that due to hypogonadism. Although testis volumes in the MP group were almost 30% smaller than in control rats, they were significantly larger in hypogonadal rats treated with GH. Conversely, rats receiving testosterone only suffered greater losses of testis volumes than rats in the MP group. Although the mechanisms behind the tropic effects of GH on testis are yet to be clarified, several clinical (18) and preclinical reports (19) suggest that GH treatments facilitate testicular growth. Our data are in agreement with studies showing that serum testosterone levels are increased by GH treatments, although testosterone treatments decreased serum IGF1 expression, potentially diminishing the associated benefits. These observations further support the use of GH as a treatment for micropenis that ameliorates the adverse effects of testosterone monotherapy on testicle growth.

Although changes in tibial lengths corresponded with changes in ratios of penile and tibial lengths, prolonged treatments with testosterone might affect bone growth. Oestrogen rather than testosterone, however, played a major role in bone metabolism and retarded bone growth when present in excess (20). Because the present rats had induced hypogonadism, anti-osteogenic effects of oestrogen are unlikely to reflect limited aromatisation of testosterone. Although high testosterone levels were observed in rats receiving exogenous testosterone, these were not sustained following administration of injectable testosterone in other studies. Thus, in the present rats, the lack of aberrant bone growth may be explained by hypogonadism and the temporary actions of exogenous testosterone. Yet, in previous studies, exogenous testosterone affected bone growth in eugonadal subjects, suggesting that assessments of tibial lengths are poorly representative of bone growth and may be insufficient to confirm the osteopathic safety of testosterone treatments. Assessing tibial bone mass might reveal the difference that was not seen in mere comparison of length. These questions will be subjects of future studies of more factors in relation to bone metabolism.

Notwithstanding the limitations of our assessments of safety, our experiments suggest that combination therapy with testosterone and GH can circumvent the problems associated with monotherapies for hypognadism. In particular, as an adjunct to testosterone, GH may enhance penile growth and preserve structural integrity.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Conception and design were conducted by Jin Kyu Oh, Kwanjin Park. Acquisition of data was done by Jin Kyu Oh, Young Jae Im. Analysis and interpretation of data were conducted by Young Jae Im, Kwanjin Park. Drafting the article was by Jin Kyu Oh, Young Jae Im. Revising it for intellectual content was by Jae Seung Paick, Kwanjin Park. Final approval of the completed article was by Jin Kyu Oh, Young Jae Im, Jae Seung Paick, Kwanjin Park.

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초 록

서론: 왜소음경의 가장 흔한 원인으로 저성선기능 자극성 성선기능저하증을 꼽을 수 있으며, 이러한 원인으로 생긴 왜소음경의 대표적인 치료법은 사춘기 시점의 남성호르몬 주사 치료로 알려져 있으나 이에 대한 대규모 연구는 전무한 실정이다.

현재까지 성장호르몬이 음경의 발달에 기여하는 효과에 대해서는 많은 연구가 이루어지지 않았으나 일부 동물실험을 통해 성장호르몬이 일정 부분 음경의 구조적인 내실화에 기여하고 남성호르몬 수용체 작용을 촉진하는 효과가 있다고 보고되었다. 이러한 연구 결과를 토대로 왜소음경의 치료에 성장호르몬 치료가 일정 부분 긍정적인 효과가 있을 것으로 기대된다.

본 연구는 동물실험을 통해 왜소음경의 치료에 있어 성장호르몬과 남성호르몬 단독 및 복합 투여했을 때 각각의 치료에 따른 음경의 길이, 두께 등의 구조적인 변화, 정강뼈의 길이 측정, 제 1형 및 3형 콜라겐의 분포, 평활근 액틴, 지방 소구체 및 동양 혈관의 밀도 등에 대하여 조사하고 이를 토대로 각각의 치료에 따른 효과를 비교 분석하여 성장호르몬 투여의 기대효과를 고찰해 보고자 하였다.

방법: 출산을 1주 앞둔 7마리의 timed-pregnant Sprague-Dawley 백서에게서 출산한 새끼를 태생 3일째에 항문-성기간 길이를 측정하여 웅성 백서만을 분리하였다. 이렇게 준비된 웅성 백서를 각각 대조군 (C), 미세음경군 (MP), 남성호르몬 단독투여군 (T), 성장호르몬 단독투여군 (G), 남성호르몬 및 성장호르몬 복합투여군 (GT)로 각각 나누었다. 이들 중

대조군을 제외한 나머지 군들은 12주령까지 매주 Leuploride acetate를 투여하여 저성선기능 자극성 성선기능저하증을 유발하였고, 성장호르몬 단독투여군 (G) 및 남성호르몬 및 성장호르몬 복합투여군 (GT)에는 성장호르몬을 이틀에 한번씩 2.5mg/kg씩 투여하였다. 남성호르몬 단독투여군 (T) 및 남성호르몬 및 성장호르몬 복합투여군 (GT)에는 매주 0.4mg/kg의 용량으로 남성호르몬을 투여하였다. 태생 12주 이후에 각 군에서 음경의 부피변화와 음경해면체 조직의 조밀한 정도를 측정하기 위해 조직학적인 표지자들을 확인하였다. 정강뼈의 길이 및 고환의 무게를 측정하였으며, 평활근 액틴, 제1형 및 3형 콜라겐, 지방 소구체의 숫자 및 동양혈관의 밀도, 남성호르몬 수용체의 발현 정도 등을 측정하였다.

결과: 대조군 (C)과 비교하여 미세음경군 (MP)에서 고환의 무게, 음경의 부피변화, 음경해면체 조직의 조밀한 정도, 평활근 액틴 및 동양혈관의 밀도, 남성호르몬 수용체의 발현 등에서 유의한 감소 소견이 관찰되었다. 반면 미세음경군의 경우 제1형 콜라겐, 제3형 콜라겐 및 지방 소구체의 숫자에서는 현저한 증가 소견을 보였다. 남성호르몬 단독투여군 (T)의 경우 일부 음경의 부피증가는 확인되었으나 성장호르몬 단독투여군 (G) 및 남성호르몬 단독투여군 (T) 모두 정상에 가까운 음경의 부피증가 소견은 관찰되지 않았으며, 남성호르몬 및 성장호르몬 복합투여군 (GT)에서만 음경의 부피증가가 정상적으로 이루어졌다. 남성호르몬 단독투여군 (T)에서는 안드로겐 수용체 발현 억제 소견이 관찰된 반면 성장호르몬을 투여한 군 (G, GT) 에서는 음경해면체내 안드로겐 수용체의 발현 촉진이 관찰되었다. 성장호르몬 단독투여군 (G)의 경우 제3형 콜라겐의 발현감소는 확인되었으나 다른 조직학적 지표에 영향을 미치지는 않았다. 남성호르몬 및

성장호르몬 복합투여군 (GT)에서만 평활근 액틴의 발현, 지방 소구체의 숫자 및 동양혈관 밀도의 증가 소견을 보였다.

결론: 본 연구 결과 성장 호르몬 및 남성호르몬 복합요법은 저성선기능 자극성 성선기능저하증과 관련된 미세음경의 크기 및 구조적인 문제점을 안전하고 효과적으로 개선할 수 있을 것으로 기대된다.

주요어: 백서, 미세음경, 남성호르몬, 성장호르몬

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